Eur. Phys. J. E **14**, 299–308 (2004)
DOI 10.1140/epje/i2004-10019-y

THE EUROPEAN PHYSICAL JOURNAL E

Tilt modulus of a lipid monolayer

S. May[1,a], Y. Kozlovsky[2], A. Ben-Shaul[3], and M.M. Kozlov[2]

[1] Junior Research Group "Lipid Membranes", Friedrich-Schiller-Universität Jena, Neugasse. 25, Jena 07743, Germany
[2] Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv 69978, Israel
[3] Department of Physical Chemistry and the Fritz Haber Research Center, The Hebrew University, Jerusalem 91904, Israel

Received 13 January 2004 and Received in final form 12 May 2004 /
Published online: 3 August 2004 –

Abstract. In addition to the familiar bending and stretching deformations, lipid monolayers and bilayers in their disordered state are often subjected to *tilt* deformations, occurring for instance in structural rearrangements accompanying membrane fusion, or upon insertion of "oblique" hydrophobic proteins into lipid bilayers. We study the elastic response of a flat lipid monolayer to a tilt deformation, using the spatial and conformational average of the chain end-to-end vector from the membrane normal to define a macroscopic membrane tilt. The physical origin and magnitude of the corresponding tilt modulus κ_t is analyzed using two complementary theoretical approaches. The first is a phenomenological model showing that the tilt and bending deformations are decoupled and the effects of inter-chain correlations on the tilt modulus is small. The second is based on a molecular-level mean-field theory of chain packing, enabling numerical evaluation of the tilt modulus for realistic, multi-conformation, chain models. Both approaches reveal that the tilt modulus involves two major contributions. The first is *elastic* in origin, arising from the stretching of the hydrocarbon chains upon a tilt deformation and reflecting the loss of chain conformational freedom associated with chain stretching. The second, purely entropic, contribution results from the constraints imposed by a tilt deformation on the fluctuations of chain director orientations. Using the chain-packing theory we compute the two contributions numerically as a function of the cross-sectional area per chain. The elastic and entropic terms are shown to dominate the value of κ_t for small and large areas per chain, respectively. For typical cross-sectional areas of lipid chains in biological membranes they are of comparable magnitude, yielding $\kappa_t \approx 0.2 k_{\rm B}T/\text{Å}^2$.

PACS. 87.16.-b Subcellular structure and processes – 61.20.Gy Theory and models of liquid structure – 61.30.St Lyotropic phases

1 Introduction

A phospholipid molecule consists of a hydrophilic polar head group and a hydrophobic tail, the latter comprising usually two hydrocarbon chains. Phospholipids, or simply lipids, differ in the chemical composition of their polar head and/or the length and degree of saturation of their hydrocarbon tails. In aqueous solution, lipids self-organize into supra-molecular assemblies of different possible geometries, depending on the constituent molecules and the ambient conditions. A common feature of all the self-assembled aggregates is that their hydrocarbon tails organize to form compact hydrophobic cores, which are effectively shielded from contact with water by the mantle of polar heads. Another structural characteristic of the aggregates, whether lipid bilayers, hexagonal phases, cylindrical aggregates as well as spherical micelles, is that they all consist of lipid *monolayers*, differently curved, depending on the spontaneous curvature of the constituent molecules [1,2]. Lipid bilayers are composed of two apposed monolayers and are of particular importance because they constitute the principal structural element (the matrix) of biological membranes.

Lipid layers under external stresses undergo elastic deformations whose magnitudes reflect the material properties of the lipid molecules. The elastic properties of lipid membranes (and other lipid phases) play important roles in ubiquitous biological and biotechnological processes [3], *e.g.*, maintenance and transformations of cell shapes [4], membrane fusion [5], membrane-mediated inter-protein interactions [6–13], and liposome formation [14].

Lipid membranes are commonly described as mathematical surfaces, analogous to Gibbs interfaces [15], characterized by elastic properties. The most familiar and extensively studied elastic modes of membranes are bending (*i.e.*, curvature) [16] and stretching (area) [17] deformations. At the same time, it has been recently realized that

[a] e-mail: Sylvio.May@uni-jena.de

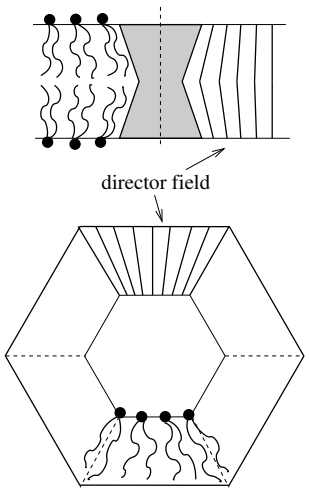

Fig. 1. Two examples of deformed lipid monolayers involving a nonzero chain tilt. Top: an hourglass-like hydrophobic inclusion such as a trans-membrane protein induces a gradually decaying tilt of its neighboring lipid chains. Bottom: a cross-section of a unit cell of the inverse-hexagonal lipid phase. Along each facet the lipid chains are concertedly tilted.

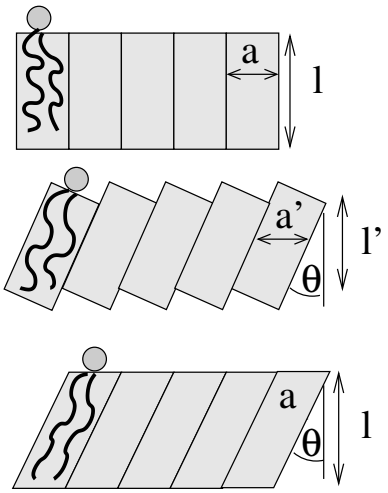

Fig. 2. Top: an unperturbed planar monolayer. The average cross-sectional area per lipid is a, and its hydrophobic thickness is l. Middle: a homogeneous deformation that involves both tilt (of tilt angle θ) and a lateral extension of the monolayer, characterized by an increase of the cross-sectional area per lipid, $a' = a/\cos\theta$. Due to the monolayer incompressibility, the hydrophobic monolayer thickness, $l' = l\cos\theta$ decreases. Bottom: a pure-tilt deformation (of tilt angle θ) that leaves a and l unaffected.

in many cases the lipid monolayers undergo another type of deformation, consisting in tilting of the hydrocarbon chains of lipid molecules with respect to the monolayer plane. This deformation, referred below to as the tilt deformation, has been shown to be generated in ubiquitous situations where the initial monolayer structure is perturbed by structural defects or membrane insertions. Among the most prominent examples of membrane configurations that require tilt deformations are (see Fig. 1) the inverted hexagonal phase [18], where chain tilt is generated by the molecular packing within the hydrophobic interstices of neighboring lipid cylinders [19], and the neighborhood of protein molecules, inserted into the lipid matrix [20, 21]. Other examples include the intermediate structure of membrane fusion and fission called membrane stalk [22–24], aqueous pores in lipid bilayers [25], ripple-like instabilities of fluid membranes [26], and the immediate lipid surrounding of oblique hydrophobic peptides [27,28]. Analysis of these structures requires accounting for the tilt elasticity of lipid monolayers.

The tilt deformation has already been considered in Helfrich's pioneering work on membrane elasticity [16], and then pursued in a series of studies devoted to the elastic properties of membranes in the *crystalline* (low temperature) state [29–31]. Here, the hydrocarbon chains are in a fully stretched ("all-trans") conformation, behaving as rigid rodlike particles in a smectic liquid-crystalline phase [32]. On the other hand, under physiological conditions all biologically relevant processes involve lipid membranes in their liquid-like state. In this state the lipid molecules are mobile within the membrane plane and the interfacial area per lipid chain is significantly larger than in the crystalline phase. Consequently, the hydrocarbon lipid tails are flexible and disordered, exploring a multitude of possible chain conformations. In the liquid state, the overall chain orientation, as measured for instance by the angle between its end-to-end vector (the "director") and the membrane normal, undergoes thermal fluctuations around its average direction. In a planar or uniformly bent (but otherwise unperturbed) liquid monolayer, the average director coincides with the membrane normal. However, in nonuniformly perturbed monolayers (like those shown in Fig. 1) the director needs not —or even cannot— point in normal direction. For example, an hourglass-like rigid membrane inclusion (see Fig. 1, top) can only be accommodated in a strictly planar bilayer if a tilt deformation takes place. Evidently, a tilt contribution must be part of an elastic description for such a case. Indeed, theoretical considerations suggest quite generally that for locally perturbed monolayers the tilt deformation may account for a significant portion of the elastic energy corresponding to various locally nonbilayer lipid structures [18,21,26,33]. This realization has inspired the formulation of some preliminary models of tilt elasticity [18,21].

Tilt deformation is deviation of the chain director from the membrane normal; see Figure 2. Generally, an elastic deformation of a monolayer may be composed of different deformation modes, such as changes in lateral membrane area, curvature, and tilt. To characterize the tilt contribution it is useful to consider a monolayer that is subject to a homogeneous tilt deformation where —on average— all chain directors deviate equally (at least locally) from the monolayer normal direction. There are two possible ways to produce such deformation, as illustrated in Figure 2. The first is to simply rotate each lipid molecule by the tilt angle, θ (Fig. 2, middle). In this case, the

deformation is accompanied by an increase of the molecular cross-sectional area, $a' = a/\cos\theta$, measured within the membrane plane. Due to the volume incompressibility of the lipid hydrocarbon moiety, this deformation is accompanied by a decrease of the monolayer thickness, l, to the value $l' = l\cos\theta$. Hence, such deformation involves membrane lateral extension (or transverse compression). The second way is to skew each lipid molecule (Fig. 2, bottom) keeping the molecular volume constant. It can be easily seen, that tilting by molecular skewing changes neither the molecular area in the membrane plane nor the monolayer thickness. Hence, it is independent from any other kind of membrane deformation and we define it as the pure tilt. Being an independent deformation, the pure-tilt deformation is described by a new material constant, κ_t, the *tilt modulus*. No direct experimental measurements of κ_t are available so far, yet its magnitude was estimated based on experimental data pertaining to the temperature-induced lamellar to inverse-hexagonal phase transition in lipid systems [18]. Previous phenomenological models for the microscopic origin of the tilt modulus suggested that the only contribution to κ_t is the stretching energy of the hydrocarbon chains upon the tilt deformation, as illustrated in Figure 2. While having the chain stretching in common with a lateral monolayer extension, the tilt deformation does not affect the region of the polar lipid head groups [18]. Therefore, the estimated values of κ_t were considerably smaller than those of the lateral stretching modulus of a monolayer. At the same time these models did not account for another important contribution to the tilt modulus, which has entropic origin and results from the loss of orientational freedom of the chain director.

In this work we theoretically calculate the tilt modulus, avoiding the approximations above, and providing a molecular-level interpretation of the mechanisms dominating κ_t. We begin by defining a macroscopic average membrane tilt, allowing the local tilt to undergo thermal fluctuations. Using a phenomenological elastic free energy we then show that the tilt modulus involves an elastic chain stretching contribution and a purely entropic contribution reflecting the loss of chain orientational freedom. Then, based on a molecular-level mean-field theory of chain packing in lipid layers we calculate the tilt modulus for lipid monolayers of different thicknesses, composed of chains of varying length. These calculations support the qualitative predictions of the phenomenological model, especially the existence of two contributions to κ_t, and provide numerical estimates of this elastic modulus.

2 Tilt free energy and elastic modulus

2.1 Chain tilt

We consider a lipid monolayer which is part of a flat lipid bilayer of hydrophobic thickness $2l$ and overall area A. The hydrophobic part of the monolayer consists of M incompressible hydrocarbon chains, each of volume $v = al$, where $a = A/M$ is the average cross-sectional area per chain in the monolayer plane. Because of its fluid-like nature, the monolayer is isotropic in the lateral directions. We define a Cartesian coordinate system such that the (x, y)-plane coincides with the hydrocarbon-water interface, thus separating the lipid head groups of the monolayer from the hydrocarbon tails. The z-axis points along the monolayer normal, towards the hydrophobic core, with $\mathbf{N}$ denoting the unit normal vector.

The hydrocarbon chains comprising a lipid monolayer in its 2D fluid state possess a multitude of different possible conformations, α. In principle, any chain conformation is fully specified by the positions of all its constituent atoms. A slightly less detailed, yet similarly satisfactory characterization is provided by the positions, $\mathbf{r}_i$, of all chain *segments*; *e.g.*, for a simple N-segment long alkyl chain (with typically $N = 12$–18 for double-chained phospholipids), $-(CH_2)_{N-1}-CH_3$ (in short C-N chain), these are the $N-1$ position vectors of the methylene groups, and the position $\mathbf{r}_t$ of the terminal methyl group. The configurational state of a monolayer composed of M chains is thus specified by the set of all α_i with $i = 1, \ldots, M$. Alternatively, the state of the monolayer may be specified by the 2D distribution of chain conformations across the (x, y)-plane $\{\alpha(\mathbf{x})\}$, with $\alpha(\mathbf{x})$ denoting the conformation of a lipid chain originating at point $\mathbf{x}$ of the monolayer-water interface.

A tilt deformation changes the probability distribution of chain conformations in the membrane, whose major manifestation is a deviation of the average chain orientation from the monolayer normal. A convenient quantitative measure of the orientation of a chain in conformation $\alpha = \alpha(\mathbf{x})$ is provided by its end-to-end vector

$$\mathbf{n}(\alpha) = \{n_x(\alpha), n_y(\alpha), n_z(\alpha)\} = \frac{\mathbf{r}_t(\alpha) - \mathbf{x}}{|\mathbf{r}_t(\alpha) - \mathbf{x}|} \qquad (1)$$

henceforth also referred to as the chain *director*. The tilt $\mathbf{t} = \mathbf{t}(\alpha)$ of a chain with respect to the membrane normal $\mathbf{N}$ will be defined as [30,34]

$$\mathbf{t} = \frac{\mathbf{n}}{\mathbf{n}\cdot\mathbf{N}} - \mathbf{N}. \qquad (2)$$

According to this definition, the tilt field $\mathbf{t} = \mathbf{t}(\mathbf{x})$ lies in the monolayer plane, with the absolute value of each tilt vector being equal to the tangent of the angle θ between $\mathbf{n}$ and $\mathbf{N}$; $|\mathbf{t}| = \tan\theta$. Figure 3 illustrates schematically a lipid chain in conformation α with corresponding director $\mathbf{n}$, tilt $\mathbf{t}$, and tilt angle θ.

2.2 The tilt modulus

We define the macroscopic tilt, $\langle\mathbf{t}\rangle$, of a deformed monolayer as the average of the chain tilt, $\mathbf{t} = \mathbf{t}(\mathbf{x})$, over the monolayer area A and over all possible conformations α of the individual chains, *i.e.*,

$$\langle\mathbf{t}\rangle = \frac{1}{A}\int \mathcal{D}\alpha(\mathbf{x}) \int\limits_A \mathrm{d}\mathbf{x}\, P[\alpha(\mathbf{x})]\, \mathbf{t}[\alpha(\mathbf{x})], \qquad (3)$$

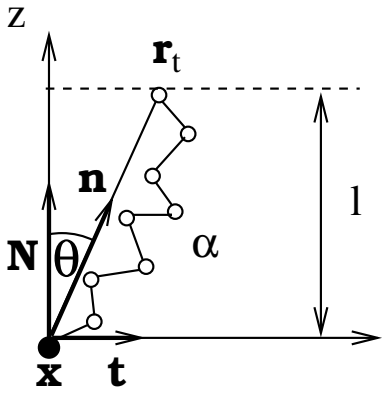

Fig. 3. A conformation α of a lipid chain and the corresponding chain director $\mathbf{n}$, defined in equation (1). Also indicated are the tilt $\mathbf{t}$ (see Eq. (2)) and the monolayer normal $\mathbf{N}$ (pointing along the z-axis). The thickness of the hydrophobic region of the monolayer is l. Note that tilt implies, on average, stretching of the chain to comply with the uniform packing properties of the monolayer. That is, for tilt angle θ the average end-to-end distance is $l/\cos\theta$.

where the integration $\int_A \mathrm{d}\mathbf{x}$ extends over the monolayer surface, and $\int \mathcal{D}\alpha(\mathbf{x})$ represents summation over all possible distributions $\{\alpha(\mathbf{x})\}$ of chain conformations within the monolayer. $P[\alpha(\mathbf{x})]$ is the probability to find the particular chain conformational distribution $\alpha(\mathbf{x})$ within the monolayer.

An undeformed planar lipid monolayer in the fluid state is isotropic in the (x, y)-plane and hence characterized by zero average tilt, $\langle\mathbf{t}\rangle_0 = 0$. We denote the free energy of this monolayer by F_0. Consider now a deformed monolayer with a nonzero average tilt $\langle\mathbf{t}\rangle$. This monolayer is no longer isotropic because $\langle\mathbf{t}\rangle$ points towards a specific direction within the (x, y)-plane. The corresponding free energy, F, up to quadratic order in the tilt can be expressed in the form

$$F = F_0 + \frac{A}{2}\,\kappa_t\,\langle\mathbf{t}\rangle^2\,, \tag{4}$$

where κ_t is the tilt modulus, reflecting the resistance of the monolayer to the tilt deformation. More generally, the tilt modulus is a two-dimensional tensor of second order. However, because the relaxed fluid monolayer is laterally isotropic this tensor is diagonal, with $\kappa_t^{xx} = \kappa_t^{yy} = \kappa_t$.

2.3 Calculating the tilt modulus

The present section provides a short derivation of the basic relationships needed for calculation of the effective tilt modulus accounting for the director fluctuations in the state of thermal equilibrium. In principle, the free energies of the deformed and relaxed monolayers, and hence its tilt modulus κ_t, can be derived by calculating the corresponding partition function Z; $F = -k_\mathrm{B}T\ln Z$, (k_B is Boltzmann's constant and T the absolute temperature). Using a continuum notation, where $h(\alpha) = h[\alpha(\mathbf{x})]$ denotes the local free-energy density corresponding to a given conformation, $\alpha = \alpha(\mathbf{x})$, of the hydrocarbon chain originating at $\mathbf{x}$, the partition function for the deformed monolayer is

$$Z = \int_{\langle\mathbf{t}\rangle} \mathcal{D}\alpha(\mathbf{x}) \cdot \exp\left\{-\frac{1}{k_\mathrm{B}T}\int_A \mathrm{d}\mathbf{x}\cdot h(\alpha)\right\}, \tag{5}$$

where the subscript $\langle\mathbf{t}\rangle$ indicates that the sum, $\int_{\langle\mathbf{t}\rangle}\mathcal{D}\alpha(\mathbf{x})$, over the various possible conformations should only include those many-chain configurations satisfying the constraint of a fixed average tilt $\langle\mathbf{t}\rangle$. This approach to calculating Z presents substantial mathematical difficulties, rendering the evaluation of κ_t impractical, even numerically.

A familiar statistical mechanical alternative which circumvents this difficulty involves the introduction of a weak, hypothetical, external field, $\mathbf{H}$, which interacts with the tilt vector, $\mathbf{t}$, to produce the required small deformation $\langle\mathbf{t}\rangle$, see *e.g.*, [35]. The interaction energy $-\mathbf{H}\cdot\mathbf{t}$ is added to the free energy $h(\alpha)$ of the monolayer, resulting in the partition function

$$\tilde{Z} = \int \mathcal{D}\alpha(\mathbf{x}) \cdot \exp\left\{-\frac{1}{k_\mathrm{B}T}\int_A \mathrm{d}\mathbf{x}\cdot[h(\alpha) - \mathbf{H}\cdot\mathbf{t}(\alpha)]\right\}. \tag{6}$$

In contrast to (5), the integration in (6) is not constrained, and extends over all possible conformations. The field, $\mathbf{H}$, must ensure that the average chain tilt should satisfy equation (3). Then

$$\langle\mathbf{t}\rangle = \frac{k_\mathrm{B}T}{A}\frac{\partial\ln\tilde{Z}}{\partial\mathbf{H}} = -\frac{\partial\tilde{F}/A}{\partial\mathbf{H}}, \tag{7}$$

where

$$\tilde{F} = -k_\mathrm{B}T\ln\tilde{Z} = F - A\mathbf{H}\cdot\langle\mathbf{t}\rangle = F + \mathbf{H}\cdot\frac{\partial\tilde{F}}{\partial\mathbf{H}} \tag{8}$$

is obtained from F by a Legendre transformation. (Note that (7) and (8) are vectorial equalities, $\langle t_x\rangle \sim -\partial\tilde{F}/\partial H_x$ etc.) The relationship between F and $\tilde{F}$ also implies

$$\frac{1}{A}\frac{\partial F}{\partial\langle\mathbf{t}\rangle} = \mathbf{H} = \kappa_t\langle\mathbf{t}\rangle\,, \tag{9}$$

where the last equality is valid in the limit of vanishing tilt (equivalently, vanishing field). Also the fluctuation in tilt

$$\langle\mathbf{t}^2\rangle - \langle\mathbf{t}\rangle^2 = \left(\frac{k_\mathrm{B}T}{A}\right)^2\frac{\partial^2\ln\tilde{Z}}{\partial\mathbf{H}^2} = -\frac{k_\mathrm{B}T}{A^2}\frac{\partial^2\tilde{F}}{\partial\mathbf{H}^2} \tag{10}$$

can be calculated from equation (6) where, again, the notation is such that $\langle t_x t_y\rangle - \langle t_x\rangle\langle t_y\rangle \sim -\partial^2\tilde{F}/\partial H_x\partial H_y$ etc. Using the last equation a simple familiar relationship (see *e.g.*, [36]) is obtained between the susceptibility,

$\chi = \partial\langle\mathbf{t}\rangle/\partial\mathbf{H}$, and the elastic tilt modulus:

$$\chi = \left(\frac{\partial\langle\mathbf{t}\rangle}{\partial\mathbf{H}}\right)_{H=0} = -\left(\frac{\partial^2\tilde{F}/A}{\partial\mathbf{H}^2}\right)_{H=0} = \frac{k_\mathrm{B}T}{A}\left(\frac{\partial^2\ln\tilde{Z}}{\partial\mathbf{H}^2}\right)_{H=0} = \frac{A}{k_\mathrm{B}T}\langle\mathbf{t}^2\rangle = \frac{1}{\kappa_t}, \tag{11}$$

where in the penultimate equality we have used equation (10), setting $\langle\mathbf{t}\rangle = 0$ for $H = 0$. The last equality, which provides the simple relationship $\chi = 1/\kappa_t = A\langle\mathbf{t}^2\rangle/k_\mathrm{B}T$ between the susceptibility χ, the tilt modulus κ_t, and the tilt fluctuations of the *unperturbed* ($\mathbf{H} = 0$) monolayer follows using equation (8).

3 Phenomenological model for the effective tilt modulus

We first compute κ_t on the basis of a phenomenological approach as suggested in [18,34]. Within this model, the energy of the monolayer state is represented by an effective elastic Hamiltonian that describes the hydrocarbon chains by the available orientations of the chain director $\mathbf{n}$. Note that this approach neglects the degeneracy related to the fact that several different chain conformations α may contribute to the state characterized by $\mathbf{n}$. Instead, the free-energy contributions of the various chain conformations α available at a given $\mathbf{n}$ are merged into an elastic Hamiltonian

$$h(\mathbf{n}) = \frac{\kappa}{2}\,(\mathrm{div}\mathbf{t})^2 + \frac{\kappa_t^0}{2}\,\mathbf{t}^2, \tag{12}$$

where the tilt $\mathbf{t}$ is related to the director $\mathbf{n}$ by equation (2). The first contribution to (12) is the energy of splay of the hydrocarbon chains [18,34]; κ denotes the monolayer bending modulus. It can also be regarded as the energy of elastic interactions between adjacent chains resulting from differences in their orientations. The second contribution to (12) results from the tilt-induced *stretching* of the lipid chains that ensures uniform packing of the chain segments within the hydrocarbon core. This elastic energy of stretching gives rise to the local tilt modulus κ_t^0.

The partition function (6) corresponding to the elastic free energy (12) is

$$\tilde{Z} = \int\mathcal{D}\mathbf{n}(\mathbf{x})\cdot\exp\left\{-\frac{1}{k_\mathrm{B}T}\int_A \mathrm{d}\mathbf{x}\cdot\left[\frac{\kappa}{2}\,(\mathrm{div}\mathbf{t})^2 + \frac{\kappa_t^0}{2}\,\mathbf{t}^2 - \mathbf{H}\cdot\mathbf{t}\right]\right\}, \tag{13}$$

where $\int\mathcal{D}\mathbf{n}(\mathbf{x})$ represents summation over all possible distributions of chain directors within the monolayer. Computation of (13) for small values of tilt (see appendix) results in

$$\tilde{Z} = \exp\left[\frac{A}{2k_\mathrm{B}T}\cdot\frac{H^2}{(\kappa_t^0 + 3k_\mathrm{B}T/a)}\right]\cdot\int\mathcal{D}\tilde{\mathbf{t}}(\mathbf{x})\exp\left\{-\frac{1}{k_\mathrm{B}T}\cdot\int_A \mathrm{d}\mathbf{x}\cdot\left[\frac{\kappa}{2}\,(\mathrm{div}\tilde{\mathbf{t}})^2 + \frac{1}{2}\,(\kappa_t^0 + 3k_\mathrm{B}T/a)\,\tilde{\mathbf{t}}^2\right]\right\}, \tag{14}$$

where the integration is performed over all possible realizations of the effective tilt $\tilde{\mathbf{t}}(\mathbf{x}) = \mathbf{t}(\mathbf{x}) - \mathbf{H}/(\kappa_t^0 + 3k_\mathrm{B}T/a)$.

An important feature of (14) is that the external field $\mathbf{H}$ is decoupled from $\tilde{\mathbf{t}}$, so that the susceptibility χ is easily calculated using equations (14, 8) and (11), yielding

$$\chi = \frac{1}{\kappa_t^0 + 3k_\mathrm{B}T/a}. \tag{15}$$

Using equations (11) and (15) the tilt modulus is thus given by

$$\kappa_t = \kappa_t^0 + 3k_\mathrm{B}T/a \tag{16}$$

and consists of two contributions. The first, κ_t^0, in accordance with the second term in (12), arises from the fact that tilted chains are, on average, further stretched out as compared to chains in an undeformed monolayer. Lipid chains, just like polymer chains, experience a loss of conformational entropy upon increasing their end-to-end distance. (Note, for example, that only one —the "all-trans"— bond sequence is available to a maximally extended polymethylene chain.) Thus, tilted chains have higher elastic free energy as compared to relaxed chains. The major contribution to this excess free energy arises from the loss of conformational entropy. It may be added that hydrocarbon chain stretching involves an energetic contribution as well, arising from the number of trans/gauche bonds along the chain. This contribution, however, is generally favorable since chain stretching results in less (higher energy) gauche conformers. We shall later refer to κ_t^0 as the elastic contribution to κ_t, being aware of the fact that it is predominantly entropic.

The second contribution to the tilt modulus, $\kappa_t^e = 3k_\mathrm{B}T/a$, is purely entropic, consistent with its proportionality to T. In the appendix we show that the transformation (valid for small tilt values) from $\mathbf{n}$ to $\mathbf{t}$ integration (see Eqs. (13) and (14)) results in the appearance of a "degeneracy" (rotational density of states) factor, $\omega(t) = 1/(1 + t^2)^{3/2} \approx \exp(-3t^2/2) = \exp[-(a/2k_\mathrm{B}T)\kappa_t^e t^2]$ per chain, in the partition function $\tilde{Z}$. For a given magnitude of t we can write $-(1/2)\kappa_t^e t^2 = (k_\mathrm{B}T/a)\ln\omega(t) = T\Delta S(t)/a$, where ΔS may be interpreted as the loss of orientational entropy experienced by a chain of tilt magnitude t. In other words, κ_t^e accounts for the constraints imposed by the tilt deformation on *orientational* fluctuations of the hydrocarbon chains. For $\langle\mathbf{t}\rangle = 0$ the directors $\mathbf{n}$ exhibit maximal orientational fluctuations, getting smaller for nonzero average tilt, $\langle\mathbf{t}\rangle \neq 0$.

Previous phenomenological models of the tilt modulus did not account for the entropic contribution, κ_t^e, to the tilt modulus [34,37]. On the other hand, a recently suggested simple model of a fluctuating director yielded only the second contribution, $3k_\mathrm{B}T/a$ [38], see also Section 4 below.

The entropic part of κ_t, corresponding to a typical cross-sectional area per chain in the liquid membrane state, $a \approx 0.30\,\mathrm{nm}^2$, is $3k_\mathrm{B}T/a \approx 0.1k_\mathrm{B}T/\text{Å}^2 \approx 35\,\mathrm{mJ/m}^2$. According to a previous estimate [34], the elastic part, κ_t^0, is smaller than $50\,\mathrm{mJ/m}^2$ but of the same order of magnitude. Hence, the "elastic" and "entropic" effects should contribute similarly to the tilt modulus. Our

results from a molecular-level chain-packing calculation presented below support this notion. A notable result of equation (16) is that the effective tilt modulus does not depend on the bending (splay) modulus, which accounts for splay deformations generated by tilt fluctuations. This means that in our approximation of small tilt, the splay deformation affects higher than quadratic order energy terms, and, hence, the related contributions can be neglected. Therefore, in order to determine the tilt modulus of an extended monolayer subject to a homogeneous average tilt, it is sufficient to neglect correlations between different lipid chains and, hence, to focus on the tilt modulus for a single chain derived in mean-field approximation. Based on a molecular chain model we shall present such a calculation in the following section.

4 Molecular-level chain-packing theory

In this section we calculate numerically the tilt modulus of the monolayer on the basis of a molecular-level mean-field theory for the conformational chain-packing statistics in amphiphilic aggregates. This theory has previously been applied to calculate conformational and thermodynamic properties of lipid assemblies of various geometries (for a review see [39]). Following a brief outline of the approach, we shall describe its application to calculate κ_t.

4.1 Free energy and conformational statistics

The principal goal of the mean-field theory used here is to derive an explicit expression for the singlet probability distribution function (pdf) of chain conformations in the monolayer. This pdf should take into account the boundary conditions associated with the particular packing geometry of the lipid molecules (*e.g.*, those of a planar or curved bilayer), and the constraints imposed on the conformational statistics of a given chain by its neighbors. Mathematically, this goal is achieved by minimizing the free energy per chain, $f = F/M$, subject to the relevant packing constraints.

Note that the probability distribution $P[\alpha(\mathbf{x})]$ factorizes on the mean-field level; $P[\alpha(\mathbf{x})] = \prod_{i=1}^{M} P(\alpha_i;\mathbf{x}_i)$, where $P(\alpha_i;\mathbf{x}_i)$ is the (local, conditional) probability to find chain i, anchored at position $\mathbf{x}_i$, in conformation α_i. Note the normalization $\sum_\alpha P(\alpha;\mathbf{x}) = 1$ at any given $\mathbf{x}$ (in the continuum limit we may omit the index i). Since the unperturbed monolayer is isotropic in the membrane plane $P(\alpha;\mathbf{x}) = P(\alpha)$ is independent of $\mathbf{x}$. $P(\alpha;\mathbf{x})$ is independent of $\mathbf{x}$ also for a homogeneously deformed, translationally invariant monolayer such as that in Figure 2, on which we focus here.

In terms of $P(\alpha)$ the (Helmholtz) free energy *per chain* is given by

$$\frac{F}{M} = \sum_\alpha P(\alpha)[\epsilon(\alpha) + k_\mathrm{B}T \ln P(\alpha)], \qquad (17)$$

where $\epsilon(\alpha)$ is the internal energy of the lipid chain in conformation α, *e.g.*, for simple polymethylene chains this is the sum of (trans/gauche) bond rotation energies. The first term is thus the average chain energy, $\langle\epsilon\rangle$. The second sum in (17) is $-TS/M$ where S/M is the conformational entropy per chain in the monolayer. Note that there is no α-*dependent* term in (17) to account for the interaction energy of the chain with its neighbors. This is because numerous experiments, as well as theory and computer simulations, indicate unequivocally that the hydrocarbon cores of lipid bilayers, monolayers, and other amphiphilic aggregates are uniformly packed with the lipid chain segments, typically with a density resembling the density of the corresponding liquid hydrocarbons [40,39]. Consequently, the cohesive energy of the hydrocarbon core is a constant, independent of the many-chain configuration, and thus cannot affect $P(\alpha)$. On the other hand, similar to isotropic liquids, short-range repulsive (excluded-volume) interactions between the chains play a crucial role in determining their packing characteristics. In our approach these interactions are reflected as *packing constraints* which $P(\alpha)$ must satisfy. The condition of *uniform chain segment density* within the hydrophobic core enables a simple mathematical expression of these constraints, as outlined below.

Let $\phi(\alpha,z)\mathrm{d}z$ denote the number of chain segments residing between z and $z+\mathrm{d}z$ when the chain is in conformation α. The condition of uniform segment density within the hydrophobic core thus implies that the *average* chain segment density at z,

$$\langle\phi(z)\rangle = \sum_\alpha P(\alpha)\phi(\alpha,z) = \bar{\phi} \qquad (18)$$

is a constant independent of z, whose value equals the average density in the core $\bar{\phi} = a/\nu$, with ν denoting the volume per segment in the (liquid-like) core and a the cross sectional area per chain at the hydrocarbon-water interface. For simple alkyl chains, $\nu = 27\,\text{Å}^3$ is the volume of a CH_2 group. Since the volume of the terminal CH_3 group is (to a good approximation) twice as large as that of a CH_2 segment ($\nu_{CH_3} \approx 2\nu$), the total volume of a saturated C-N chain is $v = \nu(N+1) = al$ and thus $\bar{\phi} = (N+1)/l$.

For the unperturbed monolayer (18) is the only relevant set of constraints on $P(\alpha)$, (apart from the trivial normalization condition). For a monolayer experiencing a uniform nonzero tilt deformation $\langle\mathbf{t}\rangle$, the conformational pdf should also satisfy

$$\sum_\alpha P(\alpha)\mathbf{t}(\alpha) = \langle\mathbf{t}\rangle\,. \qquad (19)$$

Note that the averaging in (19) is the single-chain, mean-field level expression of (3).

Minimization of the free-energy functional (17) with respect to $\{P(\alpha)\}$ subject to the constraints of uniform segment density (18), and nonzero tilt (19), yields the conformational pdf

$$P(\alpha) = \frac{1}{\tilde{z}} \exp\left\{-\frac{a}{k_\mathrm{B}T}[h(\alpha) - \mathbf{H}\cdot\mathbf{t}(\alpha)]\right\} \qquad (20)$$

with

$$ah(\alpha) = \epsilon(\alpha) + \nu \int_0^l \mathrm{d}z\, \pi(z)\, [\phi(\alpha, z) - \bar{\phi}] \qquad (21)$$

and

$$\tilde{z} = \sum_\alpha \exp\left\{-\frac{a}{k_\mathrm{B}T}\,[h(\alpha) - \mathbf{H}\cdot\mathbf{t}(\alpha)]\right\}. \qquad (22)$$

The factor a in equations (20–22) was introduced to ensure that $a\mathbf{H}\cdot\mathbf{t}$ has the units of energy, as in Section 2.

The function $\pi(z)$ appearing in $h(\alpha)$ and hence in the exponent of $P(\alpha)$ constitutes a continuous set of Lagrange multipliers, conjugate to the z-dependent constraint in (18). Its physical interpretation is that of a *lateral pressure profile*. Similarly, the field $\mathbf{H}$ in (20) is the Lagrange multiplier conjugate to the constraint of nonzero tilt, equation (19).

The evaluation of $\pi(z)$ and $\mathbf{H}$ requires the simultaneous solution of the self-consistency equations obtained by substituting (20) back into the constraints (18) and (19), *i.e.*,

$$0 = \sum_\alpha \left[\phi(\alpha, z) - \bar{\phi}\right] \exp\left\{-\frac{a}{k_\mathrm{B}T}\,[h(\alpha) - \mathbf{H}\cdot\mathbf{t}(\alpha)]\right\} \qquad (23)$$

for all z, and

$$0 = \sum_\alpha \left[\mathbf{t}(\alpha) - \langle\mathbf{t}\rangle\right] \exp\left\{-\frac{a}{k_\mathrm{B}T}\,[h(\alpha) - \mathbf{H}\cdot\mathbf{t}(\alpha)]\right\}. \qquad (24)$$

Because of the multitude of possible chain conformations α for any realistic model of the lipid chain, the evaluation of the lateral pressure profile, $\pi(z)$, and the auxiliary field, $\mathbf{H}$, must be carried out numerically. Some details pertaining to the numerical solution of the self-consistency equations (23, 24) are given in the next section.

Substituting $P(\alpha)$ from (20) into (17), we find

$$\tilde{F} = F - A\mathbf{H}\cdot\langle\mathbf{t}\rangle = -Mk_\mathrm{B}T\ln\tilde{z}. \qquad (25)$$

Thus, one possibility to evaluate the tilt modulus is to calculate $\tilde{F}$ for a series of $\mathbf{H}$ values and then determine κ_t by numerical differentiation according to (11). However, in Section 2 we have noted that a simpler and more elegant method to determine κ_t is based on computing the mean-square tilt fluctuations in the undeformed monolayer, where $\langle\mathbf{t}\rangle = 0$ and hence also $\mathbf{H} = 0$, *i.e.*,

$$\frac{1}{\kappa_t} = \chi = \frac{a}{k_\mathrm{B}T} \sum_\alpha P(\alpha)\, \mathbf{t}(\alpha)^2\,. \qquad (26)$$

This method, which we employ in the present work, requires the evaluation of $\pi(z)$ only for the undeformed ($\mathbf{H} = 0$) monolayer. In parallel with the numerical solution of the self-consistency equations (23) (with $\mathbf{H} = 0$) one can perform the averaging in (26). Note that equation (26) can be derived by performing the second derivative of $\tilde{Z} = \tilde{z}^M$ with respect to the field H (see Eq. (11)), noting that $\sum_\alpha P(\alpha)\mathbf{t}(\alpha) = 0$ because of the lateral isotropy of the monolayer in the absence of $\mathbf{H}$.

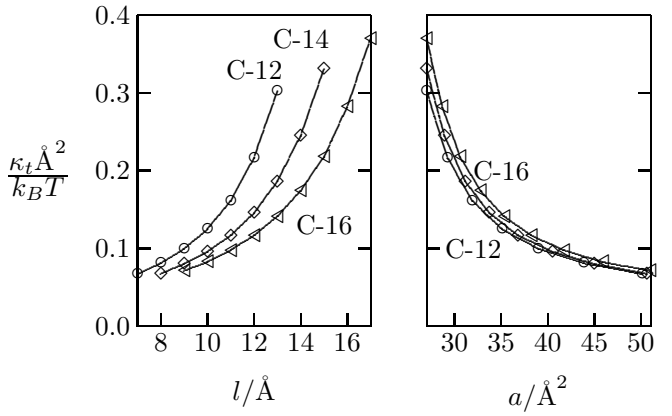

Fig. 4. The tilt modulus, κ_t, of a planar bilayer of hydrophobic half thickness, l, composed of C-12, C-14, and C-16 chains, as derived by the chain-packing theory.

4.2 Results

In our numerical calculations we use a molecular-level representation of a lipid chain according to the rotational isomeric state scheme [41]. Using this scheme, all conformations of a saturated C-N chain, consisting of N C-atoms with $N = 12, 14, 16$ were generated numerically, with each conformation α characterized by its trans/gauche (t, g_+, or g_-) bond sequence and its overall orientation with respect to the membrane normal. Bond sequences are enumerated using a bond length of 1.53 Å and the segment volumes $\nu(\mathrm{CH_2}) = 27$ Å^3 and $\nu_{\mathrm{CH_3}} = 2\nu$. For each bond sequence, 120 uniformly distributed overall chain orientations are randomly sampled, discarding all those in which one or more segments protrude into the aqueous side of the hydrocarbon-water interface.

Based on the molecular chain model we determine —for any given given cross-sectional area a per chain— the numbers of chain segments, $\phi(\alpha, z)$, that enter into the calculation of the lateral pressure. The lateral pressure is calculated according to (23) with $\mathbf{H} = 0$; as in previous work [42] we use an appropriately discretized version of (23) in which the monolayer is divided into several thin sublayers along the z-axis, each of thickness $\approx$ 1 Å. Note also that we ensure modeling of a flat and symmetric bilayer by using the mirror imaging, $\mathbf{r}_i(x, y, z) \to \mathbf{r}_i(x, y, 2l - z)$ for segments that penetrate into the apposed monolayer ($z > l$).

Figure 4 shows the tilt modulus κ_t as a function of the monolayer thickness l (left panel), and the cross-sectional area a per chain (right panel). Note that these are two alternative representations of the same set of data since $a = v/l = (N + 1)\nu/l$. The results show that, for a given area per chain a, the tilt modulus κ_t is essentially independent of chain length (N). However, κ_t depends strongly on a, especially when the chains are strongly stretched (small a). More extended chains (smaller a) exhibit a larger tilt rigidity. For large a we find that κ_t tends to become independent of a; $\kappa_t \approx 0.07 k_\mathrm{B}T/\text{Å}^2$ for $a \approx 50$ Å^2. This limiting behavior is a consequence of the fact that for large

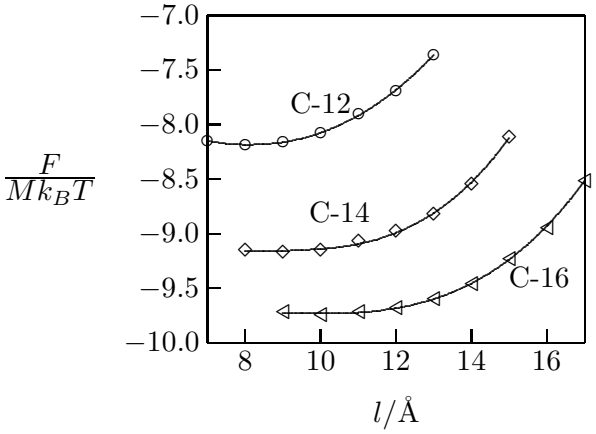

Fig. 5. The mean-field chain-packing free energy per chain, F/M, for a planar bilayer of hydrophobic half thickness, l, composed of C-12, C-14, and C-16 chains.

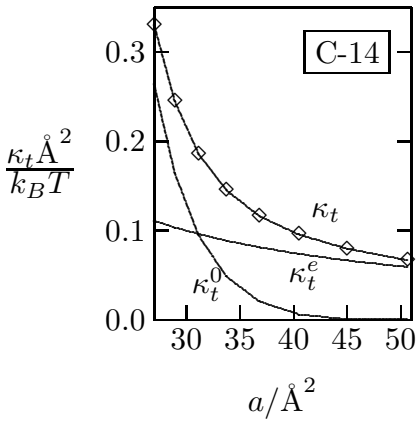

Fig. 6. The two contributions to the tilt modulus, κ_t^0 and κ_t^e, according to (29) and (30), respectively. The derivative $F'(l)/M$ in (29) is obtained from Figure 5 for C-14 chains. We also redisplay from Figure 4 the tilt modulus, κ_t, of a C-14 monolayer ($\diamond$).

a the chains are no longer stretched and the major contribution to κ_t arises from the orientational entropy term, $\kappa_t^e \approx 3k_BT/a$, which is independent of N and in the large-a region varies slowly with a.

4.3 Two contributions to the tilt modulus

The numerical results obtained from the chain-packing theory support the notion that the tilt modulus can be separated into two contributions, as suggested by (16). The following discussion elaborates on the molecular origin of these contributions and interpret them in terms of the chain-packing theory.

Recall that the first term in (16), κ_t^0 (implicit according to the phenomenological model), results from the tilt-induced stretching of the hydrocarbon chains which, in turn, is a consequence of the requirement for uniform segment density in the hydrophobic core. Upon a tilt deformation the effective end-to-end distance of the chains increases from l for vanishing tilt angle to $l/\cos\theta$ for a nonzero tilt angle θ, as follows from Figure 3. For small tilt angles we thus find the relative chain extension

$$\triangle l = \frac{\theta^2}{2}\, l. \tag{27}$$

In the tilt-deformed bilayer these (on average) elongated $l + \triangle l$ chains are packed next to each other. Ignoring momentarily the fact that the chain directors are not oriented along the membrane normal, the excess stretching free energy of the tilted monolayer may be estimated from the difference $\triangle F = F(l + \triangle l) - F(l)$. This free-energy difference, per chain, $\triangle F/M$, can be calculated based on our mean-field chain-packing theory. Thus, in Figure 5 we show $F(l)/M$ as a function of the monolayer thickness, l, for C-12, C-14, and C-16 chains. For small changes in thickness $\triangle l$ we can expand

$$F(l + \triangle l) = F(l) + F'(l)\triangle l, \tag{28}$$

where $F'(l)$ denotes the first derivative of $F(l)$. Identifying $F(l+\triangle l)$ and $F(l)$ in (28) with F and F_0 in (4), we obtain the stretching-induced contribution to the tilt modulus

$$\kappa_t^0 = \frac{l\, F'(l)/M}{a}. \tag{29}$$

Apparently, κ_t^0 would vanish for $F'(l)/M = 0$. Indeed, the curves in Figure 5 exhibit a shallow minimum which can be explained as follows: for large l the uniform packing constraint within the lipid bilayer implies stretching of the lipid chains (and a corresponding free-energy penalty). On the other hand, for very small l the chains become squeezed between the two (impenetrable) monolayer interfaces of the bilayer, again raising the free energy. We note however that the minimum of $F(l)/M$ appears for very small l (or, equivalently, large a) which is untypical for the packing of common lipid bilayers. For the physiologically relevant range of l, we find $F'(l)/M$ (and thus κ_t^0) to be positive.

The second contribution to the tilt modulus, $3k_BT/a$, can most easily be calculated on the basis of a simple director model [38]. Here, the director $\mathbf{n} = \{\cos\phi\sin\theta, \sin\phi\sin\theta, \cos\theta\}$ is allowed to change its orientations within $0 \le \phi \le 2\pi$ and $0 \le \theta \le \pi/2$. The director is thus confined to the "hydrophobic" region, $z > 0$, of the monolayer. Treating all allowed director orientations as energetically equivalent, we arrive at

$$\kappa_t^e = \frac{k_BT}{a}\,\frac{\int \mathrm{d}\mathbf{n}}{\int \mathrm{d}\mathbf{n}\,\mathbf{t}^2} = \frac{3k_BT}{a}, \tag{30}$$

where $\int \mathrm{d}\mathbf{n} = \int_0^{2\pi}\mathrm{d}\phi \int_0^{\pi/2}\mathrm{d}\theta\sin\theta$.

In Figure 6 we show κ_t^0 and κ_t^e as calculated using equations (29) and (30), respectively, and redisplay the result for κ_t from the chain-packing calculations for C-14 chains. We see that the relation $\kappa_t = \kappa_t^0 + \kappa_t^e$ is valid to a very good approximation, for the *entire* range of (biologically relevant) values of a. (Note, the minimal value

of a, corresponding to an all-trans chain, pointing along the membrane normal, is $a \approx 22$ Å; typical values of a in lipid bilayers are around 30–35 Å.) We also find that κ_t^0 dominates in the small-a region, whereas κ_t^e is more important for large a. This behavior is due to the fact that for very small a (roughly $a \lesssim 25$ Å^2) the chains are highly stretched, and any further stretching is energetically very expensive. Then κ_t^0 dominates over κ_t^e. On the other hand, for very large a (40 Å$^2 \lesssim a$) the membrane is thin, and the chains are relaxed and can easily be stretched, as evidenced by Figure 5. Thus, for large a, κ_t^0 is negligible and κ_t^e determines the tilt elasticity. For biological membranes, where typically $30 \lesssim a/$Å$^2 \lesssim 35$ [43], the two terms κ_t^0 and κ_t^e provide comparable contributions to κ_t.

5 Summary and conclusions

Our goal in this work has been to study, qualitatively and quantitatively, the elastic response of a lipid monolayer to tilt deformations. To model experimental conditions, we have characterized the monolayer state by the macroscopic tilt, $\langle \mathbf{t} \rangle$, corresponding to the conformational and spatial average of the chain tilt vector $\mathbf{t}$ in the membrane. A tilt modulus, κ_t, has been defined as the coefficient describing the (quadratic) dependence of the monolayer free energy on $\langle \mathbf{t} \rangle$. We have calculated κ_t using two theoretical approaches: the first is a phenomenological local description of the tilt elasticity [18,34], and the second is a molecular-level mean-field theory of chain packing in lipid assemblies [39]. The major results of our analysis are:

- The tilt modulus κ_t does not depend on the elastic interaction between adjacent chains resulting from a difference in their orientation. This means that, in the approximation we consider, the lipid chains fluctuate nearly independently around the average $\langle \mathbf{t} \rangle$.
- The tilt modulus κ_t consists of two contributions: an elastic one, κ_t^0, resulting from the tilt-induced stretching of the hydrocarbon chains, and an entropic contribution, κ_t^e, describing constraints imposed by tilt on fluctuations of the chain orientations.
- For large cross-sectional area per lipid chain tilt-induced chain stretching is negligible and thus $\kappa_t \approx \kappa_t^e$. On the other hand, for small cross-sectional chain areas the tilt-induced chain stretching dominates and thus $\kappa_t \approx \kappa_t^0$.
- For typical cross-sectional areas per chain ($30 \lesssim a/$Å$^2 \lesssim 35$) we find $\kappa_t^0 \approx 0.1 k_\mathrm{B}T/$Å^2 and $\kappa_t^e \approx 0.1 k_\mathrm{B}T/$Å^2 to contribute similarly to κ_t.

Hence, the present mean-field approach suggests a value of $\kappa_t \approx 0.2 k_\mathrm{B}T/$Å^2 for the tilt modulus of a typical lipid monolayer.

SM thanks the Thüringer Ministerium für Wissenschaft, Forschung und Kunst. ABS thanks the financial support of the Israel Science Foundation (grant 227/02) and the United States-Israel Binational Science Foundation (grant 2002-75). The Fritz Haber Center is supported by the Minerva Foundation, Munich, Germany. MK thanks the Human Frontier Science Program Organization, the Israeli Science Foundation (grant 75/03) and the Binational United States-Israel Science Foundation for financial support.

Appendix A. Computation of the partition function in the phenomenological model

The major technical difficulty in computing the partition function (13) arises from the fact that the Hamiltonian is a functional of the tilt field $\mathbf{t}(\mathbf{x})$, whereas the states are represented by distribution of the directors $\mathbf{n}(\mathbf{x})$ of the hydrocarbon chains. Therefore, we have to transform (13) so that the path integration is over the tilt field $\mathbf{t}(\mathbf{x})$. This transformation involves several steps:

- We replace the continuous field of the directors, $\mathbf{n}(\mathbf{x})$, by a discrete one consisting of directors of individual chains, $\mathbf{n}_i$, the area per chain in the monolayer surface being equal a and the total number of the directors being equal N.
- The path integration is then replaced by a product of ordinary integrals

$$\int \mathcal{D}\mathbf{n}(\mathbf{x}) \Rightarrow \prod_{i=1}^{N} \int \mathrm{d}\mathbf{n}_i, \tag{A.1}$$

  where, using the polar angles θ_i and ϕ_i to characterize the chain orientation, the director of a chain is determined as $\mathbf{n}_i = (\sin\theta_i \cos\phi_i, \sin\theta_i \sin\phi_i, \cos\theta_i)$ and integration over its orientations is $\int \mathrm{d}\mathbf{n}_i = \int_0^{2\pi} \mathrm{d}\phi_i \int_0^{\pi/2} \mathrm{d}\theta_i \sin\theta_i$.
- We change the variable of integration from $\mathbf{n}_i$ to $\mathbf{t}_i$. Accounting for the definition (2), the tilt of a hydrocarbon chain is presented by $\mathbf{t}_i = \tan\theta_i \cdot (\cos\phi_i, \sin\phi_i, 0)$. Using this presentation, we obtain

$$\begin{aligned} \int_0^{2\pi} \mathrm{d}\phi_i \int_0^{\pi/2} \mathrm{d}\theta_i \sin\theta_i &= \int_0^{2\pi} \mathrm{d}\phi_i \int_0^{\infty} t_i \mathrm{d}t_i \frac{1}{(1+t_i^2)^{3/2}} \\ &= \int \mathrm{d}\mathbf{t}_i \frac{1}{(1+t_i^2)^{3/2}}, \end{aligned} \tag{A.2}$$

  where $t_i = |\mathbf{t}_i| = \tan\theta_i$. As a result, we obtain

$$\begin{aligned} \prod_{i=1}^{N} \int \mathrm{d}\mathbf{n}_i &= \prod_{i=1}^{N} \int \mathrm{d}\mathbf{t}_i \frac{1}{(1+t_i^2)^{3/2}} \\ &= \left(\prod_{i=1}^{N} \int \mathrm{d}\mathbf{t}_i \right) \cdot \left(\prod_{i=1}^{N} \frac{1}{(1+t_i^2)^{3/2}} \right). \end{aligned} \tag{A.3}$$

- Within the approximation up to the second order in small tilt t_i, we replace $1/(1+t_i^2)^{3/2}$ by $\exp(-3t_i^2/2)$, insert the latter into (A.3) and using the relationship

$$\prod_{i=1}^{N} \exp\left(-\frac{3}{2} t_i^2\right) \Rightarrow \exp\left(-\frac{1}{a} \int_A \mathrm{d}\mathbf{x}\, \frac{3}{2}\, \mathbf{t}(\mathbf{x})^2\right) \tag{A.4}$$

return to the path integral over the tilt field $\mathbf{t}(\mathbf{x})$,

$$\int \mathcal{D}\mathbf{n}(\mathbf{x}) = \int \mathcal{D}\mathbf{t}(\mathbf{x}) \cdot \exp\left(-\frac{1}{a} \int\limits_A \mathrm{d}\mathbf{x}\, \frac{3}{2}\, \mathbf{t}(\mathbf{x})^2 \right). \tag{A.5}$$

The partition function (13) is now expressed in the form

$$\tilde{Z} = \int \mathcal{D}\mathbf{t}(\mathbf{x}) \cdot \exp\left\{ -\frac{1}{k_\mathrm{B}T} \int\limits_A \mathrm{d}\mathbf{x} \left[\frac{1}{2} \cdot \kappa \cdot (\mathrm{div}\mathbf{t})^2 + \frac{1}{2} \cdot \left(\kappa_t^0 + \frac{3k_\mathrm{B}T}{a} \right) \cdot \mathbf{t}^2 - \mathbf{H} \cdot \mathbf{t} \right] \right\}. \tag{A.6}$$

Changing the variable of integration from the tilt $\mathbf{t}(\mathbf{x})$ to the effective tilt $\tilde{\mathbf{t}} = \mathbf{t} - \mathbf{H}/(\kappa_t^0 + 3k_\mathrm{B}T/a)$, we obtain from (A.4) the equation (14) of the main part.

References

1. J.M. Seddon, R.H. Templer, in *Structure and Dynamics of Membranes*, edited by R. Lipowsky, E. Sackmann, Vol. **1**, section 3, second edition (Elsevier, Amsterdam, 1995) pp. 98-160.
2. V. Luzzati, in *Biological Membranes*, edited by D. Chapman (Academic Press, New York, 1968) pp. 71-123.
3. R. Lipowsky, E. Sackmann (Editors), *Structure and Dynamics of Membranes* (Elsevier, Amsterdam, 1995).
4. M. Bessis, *Living Blood Cells and Their Ultrastructure* (Springer, Berlin, 1973).
5. L.V. Chernomordik, M.M. Kozlov, Annu. Rev. Biochem. **72**, 175 (2003).
6. J.M. Park, T.C. Lubensky, J. Phys. (Paris) **6**, 1217 (1996).
7. N. Dan, P. Pincus, S.A. Safran, Langmuir **9**, 2768 (1993).
8. R.R. Netz, P. Pincus, Phys. Rev. E **52**, 4114 (1995).
9. H. Aranda-Espinoza, A. Berman, N. Dan, P. Pincus, S.A. Safran, Biophys. J. **71**, 648 (1996).
10. J.B. Fournier, Eur. Phys. J. E **11**, 261 (1999).
11. M. Goulian, Curr. Opin. Colloid Interface Sci. **1**, 358 (1996).
12. T.R. Weikl, M.M. Kozlov, W. Helfrich, Phys. Rev. E **57**, 6988 (1998).
13. S. May, A. Ben-Shaul, Phys. Chem. Chem. Phys. **2**, 4494 (2000).
14. D.D. Lasic, *Liposomes in Gene Delivery* (CRC Press, Boca Raton, 1997).
15. J.W. Gibbs, *On the Equilibrium of Heterogeneous Substances. 1876/1878*, in *The Collected Works of J. Willard Gibbs*, Vol. **1** (Yale University Press, New Haven, 1948).
16. W. Helfrich, Z. Naturforsch. **28**, 693 (1973).
17. E. Evans, R. Skalak, *Mechanics and Thermodynamics of Biomembranes* (CRC Press, Boca Raton, 1980).
18. M. Hamm, M.M. Kozlov, Eur. Phys. J. B **6**, 519 (1998).
19. R.P. Rand, N.L. Fuller, Biophys J. **66**, 2127 (1994).
20. N. Dan, S.A. Safran, Biophys. J. **75**, 1410 (1998).
21. S. May, A. Ben-Shaul, Biophys. J. **76**, 751 (1999).
22. M.M. Kozlov, V.S. Markin Biofizika **28**, 242 (1983).
23. D.P. Siegel, Biophys. J. **65**, 2124 (1993).
24. Y. Kozlovsky, M.M. Kozlov, Biophys J. **85**, 85 (2003).
25. S. May, Eur. Phys. J. E **3**, 37 (2000).
26. J.B. Fournier, Europhys. Lett. **43**, 725 (1998).
27. R.M. Epand, R.F. Epand, Biochem. Biophys. Res. Comm. **202**, 1420 (1994).
28. Y. Kozlovsky, J. Zimmerberg, M.M. Kozlov, to be published in Biophys. J. (2004).
29. W. Helfrich, J. Prost, Phys. Rev. A **38**, 3065 (1988).
30. F.C. MacKintosh, T.C. Lubensky, Phys. Rev. Lett. **67**, 1169 (1991).
31. J.V. Selinger, J.M. Schnur, Phys. Rev. Lett. **71**, 4091 (1993).
32. P.-G. de Gennes, *The Physics of Liquid Crystals* (Oxford University Press, 1987).
33. Y. Kozlovsky, M.M. Kozlov, Biophys. J. **82**, 882 (2002).
34. M. Hamm, M.M. Kozlov, Eur. Phys. J. E **3**, 323 (2000).
35. F. Reif, *Fundamentals of Statistical and Thermal Physics* (McGraw-Hill, 1965).
36. L.D. Landau, E.M. Lifshitz, *Statistical Physics* (Nauka, Moskva, 1976).
37. S. May, Eur. Biophys. J. **29**, 17 (2000).
38. K. Bohinc, V. Kralj-Iglič, S. May, J. Chem. Phys. **119**, 7435 (2003).
39. A. Ben-Shaul, in *Structure and Dynamics of Membranes* edited by R. Lipowsky, E. Sackmann, Vol. **1**, section 7 (Elsevier, Amsterdam, 1995) pp. 359-402.
40. J.N. Israelachvili, *Intermolecular and Surface Forces*, second edition (Academic Press, 1992).
41. P.J. Flory, *Statistical Mechanics of Chain Molecules* (Wiley-Interscience, New York, 1969).
42. I. Szleifer, A. Benshaul, W.M. Gelbart, J. Chem. Phys. **83**, 3612 (1985).
43. V.A. Parsegian, R.P. Rand, in *Structure and Dynamics of Membranes*, edited by R. Lipowsky, E. Sackmann, number 1B (Elsevier, Amsterdam, 1995) pp. 643-690.